Claims

1. A method for preparing fully lipid-encapsulated therapeutic agent particles of a charged therapeutic agent comprising the steps of

combining a lipid composition comprising preformed lipid vesicles, a charged therapeutic agent, and a destabilizing agent to form a mixture of preformed vesicles and therapeutic agent in a destabilizing solvent, wherein said destabilizing solvent is effective to destabilize the membrane of the preformed lipid vesicles without disrupting the vesicles,

incubating the mixture for a period of time sufficient to allow the encapsulation of the therapeutic agent within the preformed lipid vesicles, and

removing the destabilizing agent,

wherein the preformed lipid vesicles comprise a charged lipid which has a charge which is opposite to the charge of the charged therapeutic agent, and a modified lipid having a steric barrier moiety for control of aggregation, and wherein the modified lipid is present in the preformed vesicles in an amount effective to retard, but not prevent, aggregation of the preformed vesicles.

2. The method of claim 1, wherein the charged lipid in the preformed lipid vesicles comprises a cationic lipid and the therapeutic agent is an anionic therapeutic agent.

3. The method of claim 2, wherein the therapeutic agent is a polynucleotide.

4. The method of claim 2 or 3, wherein the cationic lipid is selected from the group consisting of

dioleyl-N,N-dimethylammonium chloride ("DODAC");

N-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride ("DOTMA");

N,N-distearyl-N,N-dimethylammonium bromide ("DDAB"); N-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride ("DOTAP");

3β-(N-(N',N'-dimethylaminoethane)-carbamoyl)cholesterol ("DC-Chol");

N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide ("DMRIE");

cationic liposomes comprising DOTMA and 1,2-dioleoyl-sn-3-phosphoethanolamine ("DOPE");

cationic liposomes comprising N-(1-(2,3-dioleyloxy)propyl)-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate ("DOSPA") and DOPE;

cationic lipids comprising dioctadecylamidoglycyl carboxyspermine ("DOGS") in ethanol;

N-(2,3-dioleyloxy)propyl)-N,N-dimethylammonium chloride ("DODMA") and

1,2-Dioleoyl-3-dimethylammonium-propane ("DODAP").

5. The method of any of claims 1-4, wherein the lipid composition comprises 10 to 40 mol % of the charged lipid, 25 to 40 mol % of a neutral lipid; 35 to 55 mol % of a sterol, and 2.5 to 10 mol % of the modified lipid.

6. The method of any of claims 1-5, wherein the destabilizing agent is ethanol.

7. The method of claim 6, wherein the ethanol is present in the destabilizing solvent at a concentration of 25-40 %.

8. The method of any of claims 1-5, wherein the destabilizing agent is a detergent.

9. The method of any of claims 1 to 8, wherein the destabilizing solvent further comprises 25 - 300 mM citrate buffer.

10. The method of any of claims 1 to 9, wherein the mixture is incubated at a temperature of about 40°C.

11. The method of any of claims 1-10, wherein the modified lipid is PEG-$CerC_{14}$.

12. The method of any of claims 1-11, wherein the preformed lipid vesicles comprise:

a cationic lipid,

a neutral lipid selected from the group consisting of DOPE and DSPC;

the modified lipid, and

cholesterol.

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